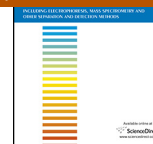




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Simultaneous extraction and determination of four different groups of pharmaceuticals in compost using optimized ultrasonic extraction and ultrahigh pressure liquid chromatography–mass spectrometry

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ABSTRACT

An analytical method for the simultaneous extraction and determination of four different groups of pharmaceuticals in compost from the biodegradation of biological infectious hazardous wastes (BIHW) was developed and successfully validated. Compost samples were spiked with known concentrations of the pharmaceuticals of interest. Ultrasonic extraction with an ethyl acetate and methanol solution (1:1) resulted to be effective for the extraction of eight target compounds. All the compounds were separated in a single gradient run by UHPLC using a Zorbax SB C18 Agilent (2.1 × 50 mm, 1.8 μm) column. Analytes were detected and quantified via multiple reaction monitoring (MRM) using an AB SCIEX API-5000TM triple quadrupole with electrospray ionization (ESI) in positive mode. The optimum mobile phase consisted of ammonium formate (2 mM, pH 3): MeOH (50:50). Recovery values of the ultrasonic extraction for all compounds were on the order of 87% to 113% with absolute deviations lower than 11%. The limits of detection and quantification for the eight pharmaceuticals were on the order of 0.66 ng g⁻¹ and 2 ng g⁻¹ respectively for all the pharmaceuticals analyzed. These values are lower than those values reported in the literature. Suitable level of linearity, acceptable limits of detection and quantification, good repeatability and inter-day precision, non-ion interference, and low matrix effect resulted from the validation of the analytical method. Implementation of the analytical procedure proposed in this research will contribute in having shorter analysis time and lower costs when working with complex matrices such as compost.

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1. Introduction

Hospitals are commonly intensive consumers of water and generators of biological infectious hazardous wastes (BIHW); moreover, hospital biological wastes (water and solids) constitute a very complex matrix, loaded with microorganisms, heavy metals, pharmaceutical products, toxic chemicals, hormonal and radioactive elements [1]. Placenta is considered one of the most frequent BIHW from hospitals and is mainly constituted of biodegradable organic matter. Thermophilic Aerobic Treatment has been used to degrade placentas as an economic and sustainable alternative, not only to transform biodegradable waste into nutrient-rich compost

that can be used as a fertilizer or soil conditioner, but also to eliminate, without risk, some organic chemicals such as emerging pollutants [2,3].

Emerging contaminants are defined as chemicals whose presence in the environment has recently been detected, and their ecological and health effects are causing growing concern. They include pharmaceuticals, personal care products, pesticides and disinfectants, among others [4]. Contamination by these compounds is still a largely unregulated area, and there is ongoing debate within the scientific community regarding which pharmaceuticals are included among the priority substances [5].

Pharmaceutically active compounds are of emerging concern because of their intrinsic biological activity, which may cause adverse effects, particularly at chronic exposure [6]. Currently, the main interest of various research groups is the development of precise and sensitive analytical methods that allow the quantification of pharmaceuticals and their metabolic and degradation products

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in different environments at low detection levels. Most of these methodologies are based on high-performance liquid chromatography (HPLC) and, recently, on tandem mass spectrometry (MS/MS) due to its high sensitivity and selectivity [6–10,18]. However, the simultaneous extraction and determination of different groups of pharmaceuticals in complex environmental matrices such as compost is particularly challenging because of the low detection limits required, the complex nature of the samples, the different chemical characteristics of the pharmaceuticals, and the difficulty in extracting and separating these compounds from interference. Recently, efforts have been conducted to develop methods for the determination of pharmaceutical compounds in sewage sludge, compost and river sediments. Methods reported are based on the ultrasonic-assisted extraction and clean-up by SPE [6]; pressurized liquid extraction (PLE) [21–25], ultrasonic solvent extraction (USE) [25–29] and microwave-assisted extraction (MAE) [30]. However, most of them have just been developed and validated for a specific sample matrix. Martín et al. [6] reported that extraction by USE is preferred because of its lower cost with respect to the other techniques reported for the extraction of pharmaceuticals from solid environmental samples, PLE and MAE. Moreover, lower degradation processes occur in USE than in PLE as extraction is carried out at room temperature instead of at high temperatures of around 100 °C reported in PLE methods and, usually, precisions higher than in MAE are achieved.

Thus, the aim of this study was to develop and validate a reliable and affordable analytical method for the simultaneous extraction and determination of four different groups of pharmaceuticals in compost obtained from the thermophilic aerobic treatment. The pharmaceuticals were two non-steroidal anti-inflammatory drugs, ketorolac and naproxen, usually administered to humans; two fluoroquinolones, ofloxacin and ciprofloxacin (which are among the most commonly prescribed class of antibiotics in Mexico); two anti-cancer (antineoplastic or cytotoxic) chemotherapy drugs, ifosfamide and cyclophosphamide; and two β -blockers, atenolol and propranolol, also called β -adrenergic blocking agents, which treat a variety of conditions, such as high blood pressure, glaucoma and migraines. The pharmaceuticals of each group were selected because they are commonly used in Mexico and environmental and health impacts has been reported. The clustering was based on the use of the drug and not on the similarity of the structure. Table 1 summarizes the characteristics of each pharmaceutical.

2. Experimental methods

2.1. Chemicals

Naproxen (NPX) sodium standard (98–100% purity), ketorolac (KET) tris salt standard ($\geq 99\%$ purity), Propranolol (PRO) hydrochloride ($>98\%$ purity), Atenolol (ATE) ($>9\%$ purity), Ifosfamide (IFO), Ofloxacin (OFL) and cyclophosphamide (CFO) monohydrate were bought from Sigma–Aldrich (Toluca, Mexico) and ciprofloxacin (CFL) ($>98\%$ purity) was purchased from Fluka Analytical (Steinheim, Germany). Methanol was acquired from J.T. Baker (Center Valley, PA, USA). Analytical grade ethyl acetate and ammonium formate were provided by Fisher Scientific (Monterrey, Mexico). Formic acid (98–100%) was from Merck (Naucalpan, Mexico). Ultrapure water was prepared with a Milli-Q water purification system (Bedford, MA, USA). A solution with methanol and ultrapure water (1:1) was prepared and labeled as Solution “A”. Then, 10 mg mL⁻¹ stock solutions for each pharmaceutical were prepared using the Solution “A” and stored at 4 °C. Standard solutions at different concentrations were daily prepared by using the stock solutions and the Solution “A”.

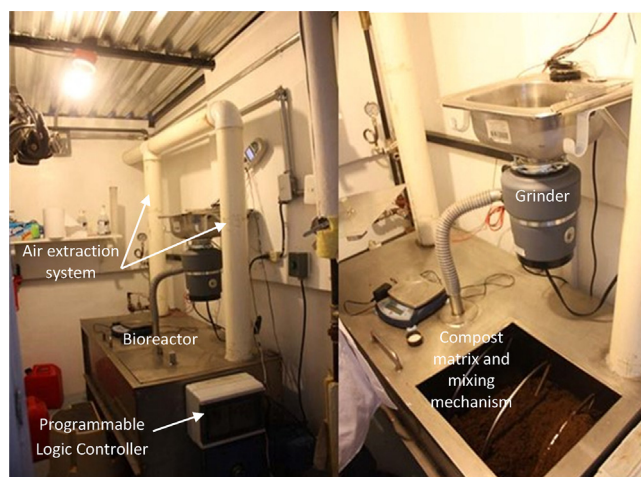


Fig. 1. Pilot thermophilic aerobic treatment system installed at the San José Hospital, Monterrey, Mexico.

2.2. Sampling of compost

Thermophilic aerobic treatment was applied in this study to transform biological infectious hazardous wastes into nutrient-rich compost that can be used as a fertilizer or soil conditioner (Fig. 1). A pilot system was installed and operated at the San José Hospital in Monterrey, Mexico. Placentas were minced with a food grinder and incorporated into the reactor of the system that uses sawdust as a matrix. The degradation process was conducted under the following conditions: matrix moisture content, 50–60%; mixing frequency, 6 times per day; and temperature, 50–60 °C. Compost samples were collected from the biological reactor and spiked with pharmaceuticals as described below.

2.3. Analytical methods

2.3.1. Samples preparation

Compost samples were transferred into centrifuge tubes and spiked with known concentrations of each pharmaceutical standard solution using micropipettes. Then, high speed vortex (1 min) was applied to homogenize the distribution and sorption of the pharmaceuticals into the compost matrix. The spiked samples were preserved at room temperature for 12 h to ensure pharmaceuticals sorption.

2.3.2. Extraction method

Volumes of 4 mL of ethyl acetate and methanol solution (1:1) were added to the compost samples (50 mg each) spiked with the eight target pharmaceuticals; next, the mixtures were vortex for 3 min and then placed into an ultrasonic bath for 5 min. Centrifugation at 3500 rpm and 4 °C, and ultra-freeze at –70 °C were conducted for 5 and 10 min, respectively. The supernatants were then decanted and evaporated with a gentle stream of nitrogen until dryness at 45 °C, and dissolved again with 500 μ L of ammonium formate (2 mM, pH 3) and methanol solution (1:1). High speed vortex for 20 s, centrifugation at 13,500 rpm and 4 °C for 5 min were applied. Then, the supernatants were filtrated through 0.2 μ m PTFE Whatman filters and the extracts were transferred to 1 mL vials for UHPLC–MS/MS analysis.

The extraction efficiency, reported as the mean recovery, was determined based on the analysis of extracts of five replicates of compost samples spiked with known concentrations of pharmaceuticals, low (6.0 ng g⁻¹), middle (80.0 ng g⁻¹) and high (180 ng g⁻¹). The coefficient of variation of the mean recovery must be lower than 15%.

Table 1

Characteristics of the pharmaceuticals used in this study.

Pharmaceutical	Systematic name	Structure	Polarity (Log P)	Molecular mass (Da)
<i>Anti-inflammatory</i>				
Ketorolac	5-benzoyl-2,3-dihydro 1H-pyrrolizine-1-carboxylic acid		2.1	255.09
Naproxen	2-(6-methoxynaphthalen-2-yl) propanoic acid		2.8	230.09
<i>Antibiotics</i>				
Ofloxacin	9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid		2.1	361.14
Ciprofloxacin	1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid		2.3	331.13
<i>Anticancer</i>				
Ifosfamide	N-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine-2-oxide		0.8	260.02
Cyclophosphamide	N,N-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide		0.8	260.02
<i>β-Blockers</i>				
Atenolol	4-(2-hydroxy-3-isopropylaminopropoxy)-phenylacetic acid		0.5	267.15
Propranolol	1-(1-methylethylamino)-3-(1-naphthyloxy)propan-2-ol		3.0	259.16

2.3.3. Ultrahigh pressure liquid chromatography and mass spectrometry method

Detection and quantification of the eight target pharmaceuticals in compost extracts were conducted by UHPLC–MS/MS analysis. The Acquity Ultra High Pressure LC-system (Waters) consisting of a degasser, binary gradient pump, auto-sampler (5 °C) and a column oven (40 °C) was used for separations. All the target compounds were separated in a single gradient run by using a Zorbax SB C18 Agilent (2.1 × 50 mm, 1.8 μm) column. The chromatographic conditions were flow rate of 250 μL min^{−1} and injection volume of 5 μL. Analytes were detected via multiple reaction monitoring (MRM) using an AB SCIEX API-5000TM triple quadrupole (Applied Biosystems/MDS SCIEX) with electrospray ionization (ESI) in positive mode. The mobile phase consisted of ammonium formate

(2 mM, pH 3) and methanol (50:50). The MS parameters were cad gas and curtain gas pressures 8 psig and 10 psig, respectively; the GS1/nebulizer and GS2/turbo GAS flow rates were 45 L min^{−1} and 30 L min^{−1}, respectively. Ion spray voltage (IS) and source temperature were fixed as 5500 V and 500 °C, respectively.

2.3.4. Method validation

Validation was done based on the guidelines of the International Conference on Harmonization for Validation of Analytical Procedures [15]. In this study, validation of the method was conducted attending the following criteria: linearity, limits of detection (LOD) and quantification (LOQ), and precision as repeatability (intraday precision) and inter-day precision. Furthermore, carryover, matrix effects and ion suppression were also evaluated.

Table 2
Recovered concentrations (RC) and mean recoveries (MR) obtained with the ultrasonic extraction method.

Spiked conc. (ng g ⁻¹)	KET RC (ng g ⁻¹) ^a	NPX RC (ng g ⁻¹) ^a	OFL RC (ng g ⁻¹) ^a	CFL RC (ng g ⁻¹) ^a	IFO RC (ng g ⁻¹) ^a	CFO RC (ng g ⁻¹) ^a	ATE RC (ng g ⁻¹) ^a	PRO RC (ng g ⁻¹) ^a
6	6.10	6.14	6.00	6.04	5.24	5.70	6.22	5.66
Std Dev	0.40	0.60	0.42	0.49	0.23	0.26	0.52	0.47
CV (%)	6.52	9.70	6.94	8.14	4.48	4.59	8.29	8.25
MR ^a (%)	101.7	102.3	100.0	100.7	87.3	95.0	103.7	94.3
80	86.10	86.24	84.34	81.18	77.80	79.24	82.68	82.86
Std	3.41	3.88	5.09	6.08	2.21	2.10	8.01	3.83
Dev CV (%)	3.96	4.50	6.03	7.49	2.84	2.64	9.69	4.63
MR ^a (%)	107.6	107.8	105.4	101.5	97.3	99.1	103.4	103.6
180	193.34	201.94	198.32	195.20	172.22	177.50	203.44	190.08
Std Dev	4.09	4.05	2.30	6.96	4.20	4.09	2.32	2.14
CV (%)	2.12	2.01	1.16	3.57	2.44	2.30	1.14	1.13
MR ^a (%)	107.4	112.2	110.2	108.4	95.7	98.6	113.0	105.6

^a Mean of five replications.

2.3.4.1. Linearity. Linearity of the calibration curves for each analyte was verified by the coefficient of determination ($R^2 > 0.98$) and the coefficient of variation between response factors ($CV < 15\%$). To generate the calibration curves, stock solutions of each pharmaceutical were prepared with Solution “A” to yield samples of five different concentrations (1 ng mL⁻¹, 10 ng mL⁻¹, 25 ng mL⁻¹, 50 ng mL⁻¹ and 100 ng mL⁻¹). 50.0 mg (dry based) compost samples were spiked with 100 µL of those solutions yielding concentrations of 2.0 ng g⁻¹, 20.0 ng g⁻¹, 50.0 ng g⁻¹, 100.0 ng g⁻¹ and 200.0 ng g⁻¹; then, the pharmaceuticals were extracted as described in the Section 2.3.2. The extracts were analyzed by UHPLC–MS/MS as described in the Section 2.3.3. Calibration curves for the eight pharmaceuticals were prepared plotting the analytical signal (peak area) versus nominal (known) concentration.

2.3.4.2. Limits of detection (LOD) and quantification (LOQ). The limits of detection and quantification were determined by analyzing extracts (by quintuplicate) of compost samples spiked with known concentration of pharmaceuticals. The LOD was established as the minimum concentration that provides a signal to noise ratio greater than three for the eight pharmaceuticals. The LOQ was set as the minimum quantifiable concentration whose average value is not deviated beyond $\pm 20\%$ ($CV < 20\%$) of the nominal (known) concentration for the eight compounds.

2.3.4.3. Precision. Precision was considered at two levels, repeatability and inter-day precision. The repeatability was obtained by quantifying the concentration of control samples at three different concentration levels, namely low 6.0 ng g⁻¹, middle 80.0 ng g⁻¹ and high 180.0 ng g⁻¹ in five replicates of the spiked compost extracts during the same day. The inter-day precision was determined by measuring on two different days, with a daily-prepared standard curve, the concentration of the control samples at three different concentration levels, namely low 6.0 ng g⁻¹, middle 80.0 ng g⁻¹ and high 180.0 ng g⁻¹ in four replicates of the spiked compost extracts. The intraday and inter-day precisions were evaluated in terms of the coefficient of variation that must be lower than 15%.

2.3.4.4. Carryover. The carryover was estimated by injecting a blank sample extract (from non-spiked compost) after injecting a spiked compost extract with a pharmaceutical concentration of 200 ng g⁻¹. The acceptance criterion was either the absence of any signal or a signal not greater than 10% of noise.

2.3.4.5. Matrix effect. The matrix effect was estimated as follows. Three extracts of non-spiked compost were obtained and then each extract was spiked by triplicate with a known concentration of each pharmaceutical at three different concentration levels, namely low (50 ng g⁻¹), middle (100 ng g⁻¹) and high (200 ng g⁻¹). Additionally,

solutions with the same three concentration levels were also prepared for each pharmaceutical. Concentrations of spiked extracts and solutions were quantified and compared. The percentage difference between corresponding determinations was calculated. There is signal enhancement if the percentage is positive and signal suppression if the percentage is negative. A value near to zero means low matrix effect.

2.3.4.6. Ion interference. The ion interference is useful to detect if one of the target compounds had some effect (ionization increase or reduction) over the others. The ion interference was determined by quantifying a pharmaceutical (by triplicate) in the presence of others. Three different concentration levels were used among the target pharmaceuticals, namely low (50 ng g⁻¹), middle (100 ng g⁻¹) and high (200 ng g⁻¹). The CV of recovered concentrations should be lower than 15%.

3. Results and discussion

3.1. Optimization of the extraction method

Nine different extraction procedures were evaluated for the eight pharmaceuticals. First, four extraction protocols developed for conventional HPLC–techniques, including solid phase extraction (SPE) and ultrasonic-assisted, and described by Martins et al. [11], Zhai and Zou [12], Christodoulou et al. [13], and Marachiello et al. [14]. When the HPLC technique was used, the Martins et al. protocol [11] was effective for the extraction of the eight pharmaceuticals; the Christodoulou et al. method [13] for the ciprofloxacin, ketorolac, naproxen and propranolol; the Maraschiello et al. procedure [14] for ofloxacin and ciprofloxacin; and the Zhai and Zou protocol [12] resulted not satisfactory for all the target compounds (results not shown in this paper). When those extraction protocols were tested with UHPLC–MS/MS, the results were not satisfactory for all the target pharmaceuticals, even for the Martins et al. method [11]. This was reasonable because such extraction methods were developed for conventional HPLC–techniques and when changing from HPLC to UHPLC analysis, MS parameters such as flow rates, ion spray voltage, source temperature, retention time and dwell times need to be altered to obtain the highest MS signals possible [16]. Due to those protocols resulted not satisfactory for all the target compounds; an exhausted work running great number of tests with different amounts of samples, extraction solvents, extraction times, extraction steps and even the use of SPE were evaluated in order to develop an optimum extraction procedure. The ultrasonic extraction method described in Section 2.3.3 resulted to be the optimum procedure for the simultaneous extraction of the eight pharmaceuticals. Table 2 presents the recovered concentrations,

Table 3

Mobile phase configurations evaluated, ions/transitions selected, and multiple reaction monitoring (MRM) conditions for the analytes detection.

Mobile phase	0.1% ammonium acetate + 0.1% acetic acid:ACN:MeOH (50:25:25)								
	Water + 0.1% formic acid:ACN (50:50)								
	MeOH + 0.1% formic acid:ACN (50:50)								
	2mM ammonium formate (pH 3):MeOH (50:50)								
Target compound	RT ^a (min)	Precursor ion (m/z)	Product ^b ion (m/z)	Molecular formula ^c	DT ^d (ms)	DP ^e (V)	EP ^f (V)	CC ^g (V)	CCEP ^h (V)
Ketorolac (KET)	2.83	255.09	105.2, 77.0, 95.1	C ₇ H ₅ O+	300	100	11	30	10
Naproxen (NPX)	4.97	230.09	185.3, 170.1	C ₁₃ H ₁₃ O+	300	115	10	30	10
Ofloxacin (OFL)	0.51	361.14	261.4, 179.1, 219.3, 221.1, 301.2, 344.2	C ₁₄ H ₁₄ FN ₂ O ₂ +	300	142	10	50	10
Ciprofloxacin (CFL)	0.55	331.13	231.2, 189.2, 203.3, 314.4	C ₁₂ H ₈ FN ₂ O ₂ +	300	150	14	50	10
Ifosfamide (IFO)	1.02	260.02	182.1, 233.3	C ₅ H ₁₀ ClNO ₂ P+	300	90	6.5	30	10
Cyclophosphamide (CPO)	1.18	260.02	140.0, 233.3	C ₄ H ₈ Cl ₂ N+	300	90	6.5	30	10
Atenolol (ATE)	0.43	267.15	145.2, 133.1, 190.0	C ₁₀ H ₉ O+	300	140	9	40	10
Propranolol (PRO)	1.11	259.16	155.2, 115.9, 128.8, 165.2, 182.9	C ₁₂ H ₁₁ +	300	130	10	40	10

^a RT: Retention time.^b Underlined value: ion transition selected for quantification purposes.^c Molecular formula of selected ion.^d DT: dwell time.^e DP: declustering potential.^f EP: entrance potential.^g CC: collision cell.^h CCEP: collision cell exit potential.

mean recoveries and the coefficient of variation of the eight pharmaceuticals obtained with the ultrasonic extraction method.

Mean recoveries were calculated as the percentage of the extracted pharmaceutical compared to the spiked level. As observed, the protocol exhibited a low variability response, with the CV ranging between 1.13% for propranolol at 180 ng g⁻¹ to 9.70% for naproxen at 6 ng g⁻¹. The recoveries were in the range of 87% and 113% for all pharmaceuticals. These recoveries are much greater than those reported by the literature for less complex matrices. Martín et al. [6] obtained recoveries of 53% for naproxen and of 16–80% for other pharmaceuticals in sediment samples. Chen et al. [17] reported poor absolute recoveries (20.7–75.4%) for 30 commonly consumed pharmaceuticals in China from samples of dewatered sewage sludge; they found that these results could be due to high matrix effects during the analytical procedure. Marsoni et al. [8] reported low recovery for ofloxacin (37%) that could be attributed to the high sensitivity of this compound to the evaporation step during their extraction method; in the ultrasonic method proposed in this study, the recovery of ofloxacin was much greater (100–110%). Shafir and Avisar [18] obtained recoveries of 11–31% for sulfonamides and tetracyclines in treated wastewater sludge and composted biosolids using tandem-solid-phase extraction cleanup and HPLC–MS/MS with an electrospray ionization. Based on those results, it is clear that the ultrasonic extraction procedure proposed in this study is an effective and reliable method to extract pharmaceuticals in complex matrices such as compost. Simplicity of the ultrasonic method developed in this study implies necessarily low cost of extraction. This is in accordance with those remarks stated by Martín et al. [6] in the sense that USE is preferred because of its lower cost with respect to the other techniques reported for the extraction of pharmaceuticals from solid environmental samples.

3.2. Optimization of the ultrahigh pressure liquid chromatography and mass spectrometry method

Four mobile phase configurations, MRM conditions and pharmaceuticals' molecules fragmentation were evaluated for the finest performance of the method. At least two ions/transitions were selected for each target analyte. But, for quantification purposes,

the most probable ion product (greater area) was selected as the quantification ion/transition and the other ions/transitions served as qualifier ions to confirm the detection of the suspected compound. Table 3 shows the mobile phase configurations evaluated and the ions/transitions selected for each compound. From several experiments conducted with compost extracts, the optimum mobile phase resulted to be the solution consisting of ammonium formate (2 mM, pH 3) and MeOH (50:50). The optimized ESI and MS/MS parameters, including precursor ions, product ions, collision energy, among others, are all summarized in Table 3.

Fig. 2 shows the final retention times obtained for each pharmaceutical and for the MRM conditions shown in Table 3. As seen, the total analysis time is much shorter than conventional HPLC techniques. The retention factor <1 and some overlapped peaks for some pharmaceuticals may suggest deficient separation; however, due to the UHPLC–MS/MS is a more sensitive method than conventional HPLC techniques, the analytical signals (peak areas) of the analytes were well detected and quantification was accurately conducted.

3.3. Validation of the method

3.3.1. Linearity

The regression equation and the coefficient of determination (R^2) for each pharmaceutical standard are shown in Table 4 for the range of 2 ng g⁻¹ to 200 ng g⁻¹. The regression equations were obtained by least-squares analysis. As seen, the coefficient of determination for all pharmaceuticals was greater than 0.98, fulfilling the validation criteria of linearity.

Table 4 also shows the recovered concentrations and the mean recoveries of the five different concentrations (five replicates each) used to generate the calibration curves for the eight pharmaceuticals under study. The coefficient of variation in all cases was found to be lower than 9.0% thus, the analytical procedure had a suitable level of linearity in terms of this parameter due to CV was lower than 15%. The eight pharmaceuticals were detected and quantified even at the lowest concentration tested (2 ng g⁻¹), with mean recoveries for all the pharmaceuticals between 94% and 104%; therefore, the method proposed in this study is reliable to analyze the target compounds in compost samples.

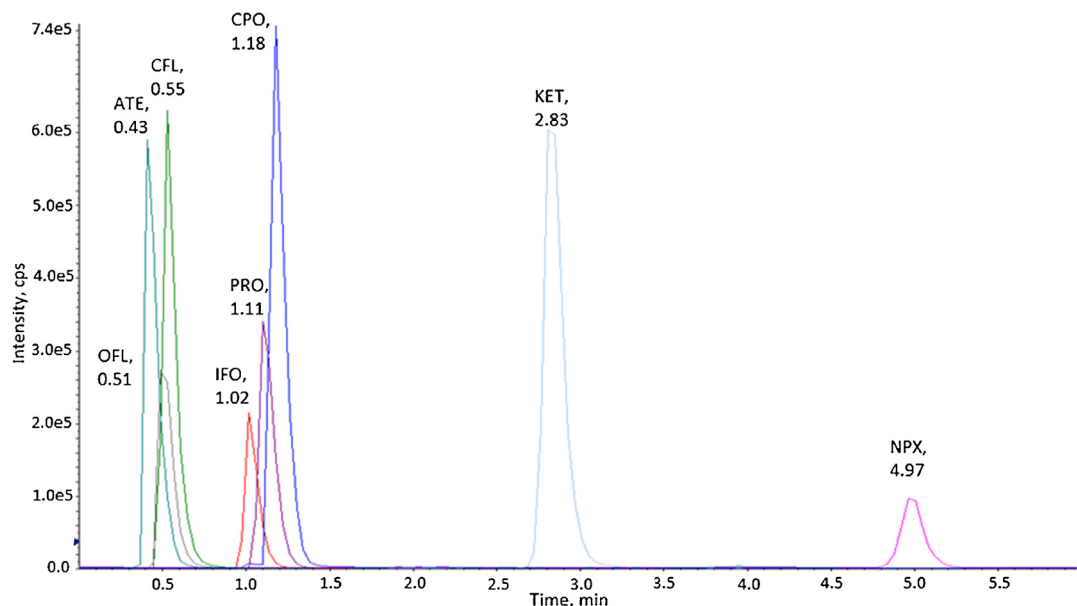


Fig. 2. Final retention times obtained for the eight pharmaceuticals studied.

3.3.2. Limits of detection (LOD) and quantification (LOQ)

A concentration of 0.66 ng g⁻¹ was set as a target LOD in order to go beyond the values reported in the literature [19]. This value (minimum concentration) provided a signal to noise ratio greater than three for the eight pharmaceuticals (Table 5). That value was set as the limit of detection of the eight pharmaceuticals for simultaneous analysis. It is clear that the limit of detection could be improved for all the pharmaceuticals because the signal to noise ratio is much greater than three. The LOD defined in this research was much lower than those values reported by the literature, 3.4 ng g⁻¹ for ofloxacin and 7.2 ng g⁻¹ for ciprofloxacin [19]; thus, the analytical method proposed in this study is suitable for the determination of these pharmaceuticals at low concentrations.

The limit of quantification for the eight pharmaceuticals investigated in this study is also presented in Table 5. As seen, the CV and the absolute deviation for all pharmaceuticals were lower than 11.0% fulfilling the acceptance criteria. On the other hand, the LOQ was much lower than those values reported in the literature for less complex matrices such as soil (11.2 ng g⁻¹ and 24.0 ng g⁻¹ for ofloxacin and ciprofloxacin, respectively) [19], sewage sludge (24.76 μg g⁻¹ for ofloxacin) [17], broiler manure (78.52 μg g⁻¹ for doxycycline) [9] and agricultural soil (1.33 μg g⁻¹ for doxycycline) [9]. Therefore, at environmentally relevant concentrations, the limit of quantification determined in this research indicates that the analytical method developed is suitable for the analyses of complex matrices such as compost.

Table 4
Mean recoveries of the five concentrations used to generate the calibration curves for the eight pharmaceuticals. Regression curves and coefficient of determination.

Spiked conc. (ng g ⁻¹)	KET RC (ng g ⁻¹) ^a	NPX RC (ng g ⁻¹) ^a	OFL RC (g ⁻¹) ^a	CFL RC (ng g ⁻¹) ^a	IFO RC (ng g ⁻¹) ^a	CFO RC (ng g ⁻¹) ^a	ATE RC (ng g ⁻¹) ^a	PRO RC (ng g ⁻¹) ^a
2	1.89	2.16	2.04	2.07	1.91	1.93	1.93	2.01
Std Dev	0.04	0.01	0.03	0.09	0.04	0.03	0.06	0.10
CV (%)	2.45	0.48	1.26	4.57	1.93	1.82	2.98	4.95
MR ^a (%)	94.5	108.0	102.0	103.5	95.5	96.5	96.5	100.5
20	20.81	18.90	20.19	20.11	20.55	20.69	20.64	20.35
Std Dev	0.12	0.26	1.03	0.24	0.62	0.45	0.35	0.09
CV (%)	0.60	1.33	5.11	1.18	3.04	2.17	1.70	0.42
MR ^a (%)	104.1	94.5	101.0	100.6	102.8	103.5	103.2	101.8
50	50.27	46.47	48.39	48.02	51.18	49.59	50.42	47.83
Std Dev	1.20	0.16	0.54	1.09	0.26	1.36	1.48	1.52
CV (%)	2.38	0.37	1.12	2.27	0.52	2.74	2.94	3.17
MR ^a (%)	100.5	92.9	96.8	96.0	102.4	99.2	100.8	95.7
100	102.72	99.04	98.97	98.45	100.54	101.78	99.73	102.64
Std Dev	2.12	3.82	5.12	8.20	1.17	3.44	1.90	4.50
CV (%)	2.06	3.86	5.18	8.33	1.17	3.38	1.91	4.39
MR ^a (%)	102.7	99.0	99.0	98.5	100.5	101.8	99.7	102.6
200	196.31	205.37	202.41	203.36	197.82	198.01	199.28	199.17
Std Dev	3.14	3.38	4.66	8.96	0.85	4.32	0.13	6.00
CV (%)	1.60	1.65	2.30	4.41	0.43	2.18	0.06	3.02
MR ^a (%)	98.2	102.7	101.2	101.7	98.9	99.0	99.6	99.6
Regression equation ^b	y = 0.9827x +	y = 1.0309x –	y = 1.0120x –	y = 1.0166x –	y = 0.9877x +	y = 0.9910x +	y = 0.9946x +	y = 0.9999x +
	0.6441	1.1510	0.4451	0.6185	0.4590	0.3356	0.2027	0.0049
R ²	0.9994	0.9993	0.9998	0.9996	0.9999	0.9998	1.0000	0.9995

^a Mean of five replicates.
^b Results are the mean of two calibration curves done in the same day.

Table 5

Limits of detection and quantification for all pharmaceuticals used in this study.

Compound	KET	NPX	OFL	CFL	IFO	CFO	ATE	PRO
LOD ^a (ng g ⁻¹)	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
(signal-to-noise ratio)	26.70	6.12	22.46	108.68	45.04	45.48	269.30	83.78
LOQ ^b (ng g ⁻¹)	1.80	2.10	2.15	2.22	2.05	1.77	1.96	1.92
Std. Dev.	0.06	0.05	0.23	0.10	0.08	0.05	0.14	0.10
CV (%)	3.42	2.56	10.53	4.30	4.04	2.67	7.01	5.31
Abs. Dev. (%)	10.22	4.77	7.58	11.19	2.34	11.32	1.84	4.22

^a Spiked concentration of 0.66 ng g⁻¹.^b Mean of five replicates. Spiked concentration: 2.0 ng g⁻¹.

3.3.3. Precision

The quality of data obtained from the analyses of pharmaceuticals in compost is the major issue of concern. Therefore, good precision, as repeatability and inter-day precision, is required to demonstrate the performance efficacy of the method. Table 6 summarizes the results of the repeatability determination for the pharmaceuticals of interest. Repeatability was obtained from the analysis, during the same day, of five replicates of the three control concentrations: low (6 ng g⁻¹), middle (80 ng g⁻¹) and high (180 ng g⁻¹). As seen, the coefficient of variation in all cases was below 10% and the mean recoveries between 87% and 108%, denoting an acceptable repeatability of the method.

Table 7 summarizes the results of the inter-day precision of the method for all the target pharmaceuticals. As explained on Section 2.3.4, inter-day precision was determined by measuring on two different days, with a daily-prepared standard curve, the concentration of the control samples at three different concentration levels, namely low 6.0 ng g⁻¹, middle 80.0 ng g⁻¹ and high 180.0 ng g⁻¹ in four replicates of the spiked compost extracts. The coefficient of variation in all analyses was below 10.22% and the mean recoveries between 87% and 108%; denoting a satisfactory inter-day precision. The results of inter-day precision were as good as those of repeatability for the eight target pharmaceuticals at the three concentration levels.

3.3.4. Carryover

The results of all tests indicated that carryover of residues in sampling/switching valves and late-eluting residues in the column were both negligible for all the pharmaceuticals analyzed (data not shown). Thus, the method performance was good for this type of complex biological matrix.

3.3.5. Matrix effect

When developing a UHPLC–MS/MS method, matrix effects are common and an important issue. The effect of co-eluting compounds arising from the matrix can result in signal enhancement or suppression [16]. Table 8 presents the results of the matrix effect evaluation. Positive values of the ME correspond to signal enhancement and negative values correspond to signal suppression. Values of the ME near to zero means low matrix effect. The matrix effect values varied from –11.12% to 6.43%, but most of the values were lower than an absolute value of 6.0% for all the target pharmaceuticals. Ciprofloxacin was the pharmaceutical with greater matrix effect. As seen, all the pharmaceuticals presented signal enhancement and suppression at different concentrations. That means that might be other factors rather than the matrix effect that deviated the measurements, particularly at the concentration 100 ng g⁻¹. Nonetheless, in general, it can be considered low matrix effect of co-extracted substances because most of the ME values are near to zero percent. These results are in agreement with those findings reported in the literature [16] which establish that matrix effects are minor or eliminated when using UHPLC–MS/MS.

3.3.6. Ion interference

In this study, the ion interference between the eight pharmaceuticals of interest was evaluated for three different concentration levels. Due to huge amount of data generated from these tests, Table 9 summarizes the coefficient of variation of recovery concentrations of each compound quantified in the presence of the others. As seen, the coefficient of variation was lower than 10.07% in all cases, which is lower than the acceptance criterion (CV ≤ 15%). This result indicates that non-ion interference occurred between the eight pharmaceuticals of interest.

Table 6

Repeatability of the analytical method for the target pharmaceuticals.

Spiked conc. (ng g ⁻¹)		KET	NPX	OFL	CFL	IFO	CFO	ATE	PRO
6	RC ^a (ng g ⁻¹)	6.10	6.14	6.00	6.03	5.24	5.70	6.22	5.67
	Std Dev	0.40	0.60	0.42	0.49	0.23	0.26	0.52	0.47
	CV (%)	6.52	9.70	6.94	8.14	4.48	4.59	8.29	8.25
	AD (%)	1.62	2.27	0.06	0.55	12.69	4.98	3.64	5.54
	MR (%)	101.7	102.3	100.0	100.5	87.3	95.0	103.7	94.5
80	RC ^a (ng g ⁻¹)	86.09	86.23	84.33	81.19	77.80	79.25	82.67	82.86
	Std Dev	3.41	3.88	5.09	6.08	2.21	2.10	8.01	3.83
	CV (%)	3.96	4.50	6.03	7.49	2.84	2.64	9.69	4.63
	AD (%)	7.62	7.79	5.41	1.49	2.75	0.94	3.34	3.58
	MR ^a (%)	107.6	107.8	105.4	101.5	97.3	99.1	103.3	103.6
180	RC ^a (ng g ⁻¹)	193.33	201.92	198.32	195.20	172.22	177.50	203.44	190.07
	Std Dev	4.09	4.05	2.30	6.96	4.20	4.09	2.32	2.14
	CV (%)	2.12	2.01	1.16	3.57	2.44	2.30	1.14	1.13
	AD (%)	7.41	12.18	10.18	8.45	4.32	1.39	13.03	5.60
	MR ^a (%)	96.7	101.0	99.2	97.6	86.1	88.8	101.7	95.0

AD: absolute deviation.

^a Mean of five replicates

Table 7
Inter-day precision of the analytical method for the target pharmaceuticals.

Spiked conc. (ng g ^{−1})		KET	NPX	OFL	CFL	IFO	CFO	ATE	PRO
6	RC ^a (ng g ^{−1})	6.50	5.35	6.36	6.27	5.90	6.11	5.61	6.19
	Std Dev	0.37	0.17	0.23	0.52	0.44	0.37	0.30	0.39
	CV (%)	5.70	3.25	3.61	8.31	7.53	6.10	5.40	6.34
	AD (%)	8.41	10.79	6.03	4.57	1.73	1.90	6.57	3.19
	MR ^a (%)	108.3	89.2	106.0	104.5	98.3	101.8	93.5	103.2
80	RC ^a (ng g ^{−1})	84.91	82.47	80.41	81.30	79.07	80.66	75.62	83.37
	Std Dev	2.98	4.10	6.01	5.64	3.03	3.21	4.31	4.34
	CV (%)	3.51	4.97	7.48	6.94	3.83	3.99	5.70	5.26
	AD (%)	6.13	3.09	0.52	1.62	1.16	0.82	5.47	4.21
	MR ^a (%)	106.1	103.1	100.5	101.6	98.8	100.8	94.5	104.2
180	RC ^a (ng g ^{−1})	186.47	195.20	181.30	186.73	174.20	180.02	184.12	186.86
	Std Dev	7.20	11.46	13.03	19.08	4.34	3.78	17.31	5.66
	CV (%)	3.86	5.87	7.19	10.22	2.49	2.10	9.40	3.03
	AD (%)	3.60	8.45	0.72	3.74	3.22	0.01	2.29	3.81
	MR ^a (%)	93.2	97.6	90.7	93.4	87.1	90.0	92.1	93.4

AD: absolute deviation.
^a Mean of four replicates.

Table 8
Matrix effect based on three different concentrations.

	KET	NPX	OFL	CFL	IFO	CFO	ATE	PRO
NC (ng g ^{−1}) ^a	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
SSC (ng g ^{−1}) ^b	51.12	46.35	48.78	48.79	51.36	50.55	49.37	48.90
SEC (ng g ^{−1}) ^c	49.42	46.59	48.01	47.25	50.99	48.63	51.47	46.76
ME (%)	−3.31	0.52	−1.57	−3.16	−0.73	−3.80	4.24	−4.39
NC (ng g ^{−1})	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
SSC (ng g ^{−1})	104.21	101.74	102.59	104.24	101.37	104.21	101.07	105.83
SEC (ng g ^{−1})	101.22	96.33	95.35	92.65	99.71	99.35	98.38	99.46
ME (%)	−2.87	−5.32	−7.06	−11.12	−1.64	−4.67	−2.66	−6.02
NC (ng g ^{−1})	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
SSC (ng g ^{−1})	194.09	202.97	199.11	197.02	197.22	194.95	199.19	194.92
SEC (ng g ^{−1})	198.53	207.77	205.70	209.69	198.43	201.06	199.37	203.42
ME (%)	2.29	2.36	3.31	6.43	0.61	3.13	0.09	4.36

^a NC: nominal concentration.
^b SSC: measured standard solution concentration.
^c SEC: measured spiked extract concentration.

As shown in previous sections, all the acceptance criteria of the International Conference on Harmonization for Validation of Analytical Procedures [15] were fulfilled satisfactory; therefore, the analytical method developed and validated in this paper is reliable for accurate measurements of different groups of pharmaceuticals in complex matrices such as compost when UHPLC–MS/MS is used and concentrations are on the order of ng g^{−1} of dry compost.

It is important to remark that the guidelines of the International Conference on Harmonization for Validation of Analytical Procedures are commonly used for product purity tests in pharmaceutical industry rather than for the environment monitoring; however, some tests are similar in validation [20]. Conventional analytical methods for environment monitoring purpose commonly use internal standards or surrogates to get accuracy;

Table 9
Coefficient of variation (%) of recovery concentrations of each compound quantified in the presence of the others.

Quantified compound	SC ^a		In presence of							
			KET	NPX	OFL	CFL	IFO	CFO	ATE	PRO
KET	200.0	SC	–	200.0	200.0	200.0	200.0	200.0	200.0	200.0
		CV		9.03	2.65	3.55	3.67	1.55	3.29	6.07
NPX	100.0	SC	100.0	–	100.0	100.0	100.0	100.0	100.0	100.0
		CV	2.52		5.23	6.22	0.06	0.23	2.80	9.37
OFL	100.0	SC	100.0	100.0		100.0	100.0	100.0	100.0	100.0
		CV	3.51	7.70	–	3.55	3.83	1.08	0.20	6.79
CFL	50.0	SC	50.0	50.0	50.0		50.0	50.0	50.0	50.0
		CV	0.54	10.07	6.09	–	1.50	2.17	0.58	5.73
IFO	100.0	SC	100.0	100.0	100.0	100.0		100.0	100.0	100.0
		CV	0.65	1.75	6.61	6.62	–	9.05	1.53	2.84
CFO	100.0	SC	100.0	100.0	100.0		100.0		100.0	100.0
		CV	3.51	7.70	3.83	3.55	1.08	–	0.20	6.79
ATE	100.0	SC	100.0	100.0	100.0	100.0	100.0	100.0		100.0
		CV	3.65	0.11	4.00	9.08	1.72	1.11		0.52
PRO	100.0	SC	100.0	100.0	100.0	100.0	100.0	100.0		–
		CV	4.40	0.73	0.76	3.75	0.05	2.89	0.44	

CV: Coefficient of variation (%).
^a SC: Spiked concentration (ng g^{−1}).

nevertheless, they are time consuming and increase the cost of the analysis. Furthermore, the validation procedures of the ICH HARMONISED TRIPARTITE GUIDELINE do not consider them because this methodology includes parameters to evaluate the extraction efficiency, the matrix effect and the ion interference. With the analytical method proposed in this study, analysis time and costs are reduced and an acceptable accuracy is achieved. Undoubtedly more research is needed to improve the chromatographic separation and validate the proposed analytical method for other pharmaceuticals and other matrices. Nonetheless, the protocol proposed in this study could be applied for pharmaceuticals with similar structure and positive ionization. As seen, most of the pharmaceuticals used in this study were different in structure because the selection was based on the use of the drug and not on the similarity of the structure; however, the ionization of all of them was in positive mode and consequently were detected and quantified simultaneously. This is an important issue to take into consideration in the future application of the protocol developed in this study.

This manuscript deals with the development and validation of an extraction, detection and quantification method via the application of the ICH HARMONISED TRIPARTITE GUIDELINE criteria, rather than application of the method to evaluate the presence of the target pharmaceuticals in compost. The application of the method was out of the scope of this paper. However, it is important to remark that the compost used in this study was free of such pharmaceuticals and their metabolites. The thermophilic aerobic biodegradation is an effective process not only to degrade biological infectious hazardous wastes, but also, to degrade micropollutants such pharmaceuticals and hormones contained in placentas and in other pathological wastes from hospitals.

4. Conclusions

An UHPLC–MS/MS analytical method for the simultaneous extraction and determination of four different groups of pharmaceuticals in compost from biological infectious hazardous wastes was developed and validated. The method fulfilled the validation criteria of the International Conference on Harmonization for Validation of Analytical Procedures. Suitable level of linearity, acceptable limits of detection and quantification, good repeatability and inter-day precision, non-ion interference, and negligible carryover and matrix effect were obtained. Ultrasonic extraction resulted to be an effective method for the simultaneous extraction of the eight pharmaceuticals analyzed. High recoveries, greater than those reported on the literature for similar matrices were obtained (87–113%, for all the compounds). The UHPLC–MS/MS analytical procedure proposed was sensitive and effective to detect and quantify simultaneously all the pharmaceuticals. The limit of detection of the method (0.66 ng g^{-1} for all the pharmaceuticals) was lower than those values reported by other researchers for similar and simpler matrices. The LOQ was on the order of 2 ng g^{-1} for all the pharmaceuticals, much lower than those values reported in the literature for less complex matrices such as soil (11.2 ng g^{-1} and 24.0 ng g^{-1} for ofloxacin and ciprofloxacin, respectively), sewage sludge ($24.76 \text{ } \mu\text{g g}^{-1}$ for ofloxacin), broiler manure ($78.52 \text{ } \mu\text{g g}^{-1}$ for doxycycline) and agricultural soil ($1.33 \text{ } \mu\text{g g}^{-1}$ for doxycycline).

Regarding the application of the analytical method, in the context of routine work at any environmental laboratory, its implementation will contribute in having suitable compounds recovery, acceptable levels of detection and quantification, minimum matrix effect, shorter analysis time and lower costs when working with complex matrices such as compost.

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